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Using multiple natural tags provides evidence for extensive larval dispersal across space and through time in summer flounder

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Abstract

Dispersal sets the fundamental scales of ecological and evolutionary dynamics and has important implications for population persistence. Patterns of marine dispersal remain poorly understood, partly because dispersal may vary through time and often homogenizes allele frequencies. However, combining multiple types of natural tags can provide more precise dispersal estimates, and biological collections can help to reconstruct dispersal patterns through time. We used single nucleotide polymorphism genotypes and otolith core microchemistry from archived collections of larval summer flounder (*Paralichthys dentatus*, n = 411) captured between 1989 and 2012 at five locations along the US East coast to reconstruct dispersal patterns through time. Neither genotypes nor otolith microchemistry alone were sufficient to identify the source of larval fish. However, microchemistry identified clusters of larvae (n = 3–33 larvae per cluster) that originated in the same location, and genetic assignment of clusters could be made with substantially more confidence. We found that most larvae probably originated near a biogeographical break (Cape Hatteras) and that larvae were transported in both directions across this break. Larval sources did not shift north through time, despite the northward shift of adult populations in recent decades. Our novel approach demonstrates that summer flounder dispersal is widespread throughout their range, on both intra- and intergenerational timescales, and may be a particularly important process for synchronizing population dynamics and maintaining genetic diversity during an era of rapid environmental change. Broadly, our results reveal the value of archived collections and of combining multiple natural tags to understand the magnitude and directionality of dispersal in species with extensive gene flow.

KEYWORDS

biological collections, dispersal, otolith microchemistry, population assignment, single nucleotide polymorphisms

1 | INTRODUCTION

Dispersal sets the fundamental scales over which ecological and evolutionary dynamics of populations occur. Dispersal drives connectivity, or the exchange of individuals among populations (Cowen & Sponaugle, 2009), and the degree of connectivity influences population dynamics (Gotelli, 1991; Hanski & Gilpin, 1997; Huffaker, 1958; Runge, Runge, & Nichols, 2006), community composition (Connolly, Menge, & Roughgarden, 2001), evolution (Slatkin, 1987; Wright, 1931), persistence (Botsford, Hastings, & Gaines, 2001; Hastings & Botsford, 2006) and management strategies (Fogarty & Botsford, 2007). Yet, understanding dispersal in the marine realm is challenging, especially because dispersal may vary over time (Nanninga & Berumen, 2014; Reis-Santos et al., 2013) and may homogenize allele frequencies (Gleason & Burton, 2016; Sandoval-Castillo, Robinson, Hart, Strain, & Beheregaray, 2018). Our understanding of dispersal through time is often limited by our ability to sample across relevant seasonal, interannual or decadal scales. Fortunately, natural history collections provide powerful, underappreciated and often underutilized opportunities to retrospectively study biological diversity in populations of interest (Johnson et al., 2011; Pimm et al., 2015; Schwartz, Luikart, & Waples, 2007). As unique repositories of life on Earth, natural history collections preserve individuals and their natural markers across space and time (Watanabe, 2019). These specimens are particularly useful for investigating a wide range of ecological and evolutionary processes (Holmes et al., 2016; Webster, 2018), especially during an era of rapid environmental change (Meineke, Davies, Daru, & Davis, 2019).

A variety of natural markers have been used to study the extent and rate of exchange between populations (Thorrold et al., 2002). Assignment methods using genetic markers have been developed to determine the most likely population an individual or a group of individuals belongs to, or to exclude individuals of interest from potential populations of origin (see review by Manel, Gaggiotti, & Waples, 2005). Genetic assignment methods (Cornuet, Piry, Luikart, Estoup, & Solignac, 1999; Paetkau, Calvert, Stirling, & Strobeck, 1995; Paetkau, Slade, Burden, & Estoup, 2004; Pritchard, Stephens, & Donnelly, 2000; Rannala & Mountain, 1997) have been used to ascertain population membership or infer dispersal between populations of fishes (Glover, Skilbrei, & Skaala, 2008; Nielsen et al., 2012; Nielsen, Hansen, Schmidt, Meldrup, & Grønkjaer, 2001; Primmer, Koskinen, & Piironen, 2000; Shaklee, Beacham, Seeb, & White, 1999), birds (Claramunt & Wright, 2018; Townsend & Navarro-Siguenza, 2018), reptiles (Berry, Tocher, & Sarre, 2004), polar bears (Paetkau et al., 1995), deer (Frantz et al., 2006) and humans (Rannala & Mountain, 1997). While genetic assignment has most often been used with putatively neutral loci, using non-neutral (candidate) loci that are more spatially diverged can be particularly useful in species with high rates of gene flow (Freamo, O'Reilly, Berg, Lien, & Boulding, 2011; Nielsen et al., 2012; Nielsen, Hemmer-Hansen, Larsen, & Bekkevold, 2009).

Connectivity and dispersal studies using genetic markers have clearly been informative, but recently, approaches that utilize multiple types of markers have highlighted complementary results on different timescales or illuminated otherwise hidden patterns (Barton et al., 2018; Bradbury, Campana, & Bentzen, 2008; Papetti et al., 2013; Reis-Santos et al., 2018; Tanner, Pérez, Presa, Thorrold, & Cabral, 2014). A number of marine dispersal studies in particular have started to combine genetics with microchemistry, another type of natural marker. Otoliths (fish ear stones), statoliths (related structures in invertebrates) and shells are structures that grow over an individual's lifetime, are metabolically inert once deposited, and incorporate trace elements into their inorganic ($CaCO_3$) and organic matrices (Thorrold, Zacherl, & Levin, 2007). Thus, microchemistry reflects the site-specific environmental characteristics of ambient waters in which each individual resided, starting with the natal core that is formed upon fertilization at the spawning and hatching site (Thorrold, Jones, & Campana, 1997). Microchemistry can be used to retroactively detect residency and movement within or between estuarine and marine systems provided that spatial gradients in temperature, salinity or water chemistry exist (Gillanders & Kingsford, 1996; Schaffler, Reiss, & Jones, 2009; Thorrold, Latkoczy, Swart, & Jones, 2001; Vasconcelos et al., 2008). However, unlike genetic markers that can integrate over multiple generations to offer a deeper historical perspective (Lowe & Allendorf, 2010), microchemistry data within otoliths are limited to individual lifespans (Gillanders, 2002). Combined approaches using genetics and microchemistry promise to improve our understanding of dispersal, but studies have generally analysed these data sets in parallel rather than in a truly integrated framework (Bradbury et al., 2008; Papetti et al., 2013; Barton et al., 2018; but see Tanner et al., 2014 & Reis-Santos et al., 2018).

Many recent studies of marine larvae have demonstrated that dispersal is more constrained than previously imagined (Almany et al., 2017; Baetscher et al., 2019; Jones, Planes, & Thorrold, 2005). Larval dispersal may be particularly limited around biogeographical breaks, such as Cape Hatteras in North Carolina. Cape Hatteras has been found to be an important barrier to larval dispersal for a variety of invertebrates and fish (Baker, Austin, Bowen, & Baker, 2008; Roy, Quattro, & Greig, 2012) because the Gulf Stream transports larvae offshore and because its divergence results in a steep thermal gradient (Briggs, 1974).

One species with a distribution straddling Cape Hatteras and that is thought to experience limited dispersal across Cape Hatteras (Kraus & Musick, 2001; Wilk, Smith, Ralph, & Sibunka, 1980) is summer flounder (*Paralichthys dentatus*). The directionality and magnitude of larval summer flounder dispersal remains unknown. Summer flounder inhabit waters from Nova Scotia, Canada, to Florida, USA (Packer et al., 1999). Relatively homogeneous allele frequencies at most loci suggest substantial dispersal throughout this range, although candidate loci under spatially divergent selection have also been identified (Hoey & Pinsky, 2018; Jones & Quattro, 1999). Larval summer flounder are spawned over the continental shelf, with the majority occurring between Cape Cod, Massachusetts, and Cape Lookout, North Carolina (Smith, 1973), during the autumn and early winter when adults move offshore (Able & Fahay, 2010). It

is unknown whether more specific spawning grounds exist. Larval summer flounder develop in the coastal ocean, but ingress to estuaries soon before settling down to their juvenile habitat, a process that is thought to take ~30 days in ambient spring temperatures (Keefe & Able, 1993). Ingressing larvae have been collected and archived at sites throughout the species range since the 1980s. With archived specimens and allele frequency differences along the coast at candidate loci, summer flounder offer an ideal opportunity to test the use of multiple natural markers to assign larvae back to their natal origins over 24 years.

In this study, we combine double-digest restriction-site associated DNA sequencing (ddRADseq) and otolith core microchemistry data on collections of larval summer flounder from 1989 to 2012 to investigate natal origins and dispersal over time. We ask: (a) Do larval summer flounder exhibit regional genetic population structure and has it remained stable over a quarter of a century? (b) How do elemental signatures from the natal core of larval otoliths differ across space and time? (c) Does combining genetic and otolith markers improve our understanding of the extent to which larval dispersal has varied across space and through time?

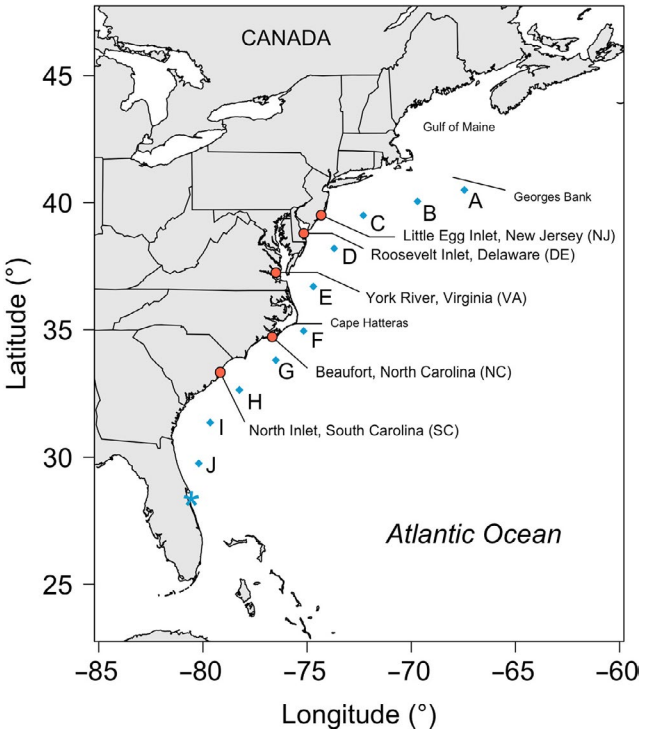

FIGURE 1 Locations of larval summer flounder (n = 411; circles) sampled from ingress sites along the US East coast between 1989 and 2012. Locations A–J denote spawning reference locations to which larvae were assigned. These locations represent distances along the coast calculated from a southern point (*) [Colour figure can be viewed at wileyonlinelibrary.com]

2 | MATERIALS AND METHODS

2.1 | Larval collections and curation

To explore regional patterns of larval population structure and connectivity throughout the majority of the species' range, we utilized several ongoing larval ingress survey and collection programmes along the US East coast (Figure 1; Table 1). We primarily obtained larvae collected at the Rutgers University Marine Field Station (RUMFS; Little Egg Inlet, New Jersey) and the National Oceanic & Atmospheric Administration's Beaufort (North Carolina) Laboratory starting in 1989. At both ingress locations, ichthyoplankton were collected from a bridge during night-time flood tides on a weekly basis and sorted to species (see Able et al., 2011; Sullivan, Able, Hare, & Walsh, 2006 for sampling protocol). To ensure adequate sample size for this study, larvae were pooled by month and 50 larvae were assembled from each of three time periods: 1989–1993, 1998–2002 and 2008–2012. These time periods are hereafter referred to as early, middle and late, respectively. To sample from the peak ingress periods for New Jersey (NJ) and North Carolina (NC), approximately five larvae were selected from winter (January–March) and five from autumn (October–December) for each year. Additional ingressing summer flounder larvae were obtained from collections taken at Roosevelt Inlet, Delaware (DE; 2008–2010), York River, Virginia (VA; 2008–2010), and North Inlet, South Carolina (SC; 2008), to extend the spatial sampling of this species' range. Larvae from these additional sites were taken from both winter and autumn periods, when possible, to match the collection periods in New Jersey and North Carolina; Virginia and Delaware specimens were among those reported in Ribeiro et al. (2015). Virginia specimens are catalogueed in the Nunnally Ichthyology Collection at the Virginia Institute of Marine Science (VIMS Catalog Numbers 19445–19494). All larval summer flounder (n = 411) had been stored in 95% ethanol at their respective institutions.

2.2 | Population structure analyses

Methods for obtaining genotypes at 1,904 loci across 293 larval individuals are detailed in the Appendix S1. We performed principal component analysis (PCA) using the ADEGENET version 2.0.1 (Jombart, 2008) and ADE4 version 1.7–10 (Chessel, Dufour, & Thioulouse, 2004) packages in R v.3.3.3 (R Core Team, 2017). We then sorted individuals into regions (collected north or south of Cape Hatteras, NC) and time periods (early, middle and late) and performed two hierarchical analyses of molecular variance (AMOVAs) using the ADE4 method of the *poppr.amova* function in the POPPR version 2.4.1 package (Kamvar, Tab ima, & Grünwald, 2014) in R with 1,000 permutations each. The first AMOVA tested for differences in genetic variance among time periods (nested within regions) and differences between regions. The second AMOVA examined differences between regions (nested within time periods) and differences between time periods (Excoffier, Smouse, & Quattro, 1992). Weir and Cockerham's pairwise F_{ST} was also calculated using the *pairwise.WCfst* function in the HEIRFSTAT version 0.04–22 package (Goudet, 2005) in R for each pair of

TABLE 1 Sampling years, source and sample size for each sampled collection or data set of larval summer flounder from north to south. See Figure 1 for locations

Year(s)	Source	$n_{sampled}$	$n_{otolith}$	$n_{genetic}$	$n_{otolith\ \&\ genetic}$
1989–1993	Little Egg Inlet, NJ	51	7	8	4
1998–2002	Little Egg Inlet, NJ	50	32	38	26
2008–2012	Little Egg Inlet, NJ	50	34	6	4
2008–2010	Roosevelt Inlet, DE	50	41	50	41
2008–2010	York River, VA	50	25	44	23
1989–1993	Beaufort, NC	55	17	52	17
1998–2002	Beaufort, NC	54	25	49	21
2008–2012	Beaufort, NC	50	16	45	15
2008	North Inlet, SC	1	0	1	0
	Total	411	197	293	151

unique ingress site and time period groups. These analyses tested how genome-wide allele frequencies differed on average across space and time.

Next, we used STRUCTURE version 2.3.4 (Pritchard et al., 2000) to determine the number of putative populations. We ran STRUCTURE using all 1,904 loci and a burn-in of 10,000 iterations followed by 200,000 Markov chain Monte Carlo (MCMC) steps assuming admixture and correlated allele frequency models with prior information on sampling location and time period. We ran 10 replicates of *K* from 1 to 5, where *K* is the number of population clusters, and we checked for parameter stabilization. We also ran STRUCTURE with the 1,646 remaining loci after removing 258 loci not in Hardy–Weinberg proportions (HWP; $p < .01$, exact test, PEGAS version 0.10 package), but the results were effectively identical and are not further discussed.

To determine the optimal number of clusters, the 10 replicates for each *K* were input into STRUCTURE HARVESTER (Earl & VonHoldt, 2012) and visualized with CLUMPP (Jakobsson & Rosenberg, 2007) and DISTRUCT (Rosenberg, 2004). Based on previous work (Hoey & Pinsky, 2018), we expected difficulty determining the optimal *K* and so we used both the mean likelihood value (L(*K*)) and Δ*K* (Evanno, Regnaut, & Goudet, 2005).

2.3 | Genetic assignment of individual larvae

As previously reported in Hoey and Pinsky (2018), 15 of 1,137 loci in adult summer flounder were found to be associated with distance along the coast, depth, bottom temperature and/or bottom salinity, and exhibited allele frequency differences along the coast. Of the 15 candidate loci previously identified in adults, 10 passed our filtering criteria in larval fish as described in "Bioinformatics & genotyping" (Appendix S1). Generalized additive models (GAMs) with a binomial error structure were fit for each of these 10 candidate loci to relate individual allele counts in adults to distance along the coast. We used the *predict.gam* function in the MGCV package (Wood, 2011) in R to predict allele frequencies of our candidate loci at 10 equidistant reference locations across the adult summer flounder sampling range (Figure 1; Figure S1). These GAM-determined allele frequencies formed the "genetic map" to which larval summer flounder were assigned.

We used these 10 loci to determine the assignment accuracy of different sized larval clusters. We simulated groups of one, five, 10, 20 or 30 diploid individuals from each of the 10 potential spawning reference locations based on allele frequencies at the 10 candidate loci (Table S2) using a custom R script. We did this 1,000 times and assigned (Paetkau et al., 1995) each simulated individual or group of individuals to the most likely reference location using a custom R script employing equation 10 of Rannala and Mountain (1997). We then examined the percentage of correct assignments.

To calculate individual genetic assignment, we calculated the genotype likelihood for each observed individual using the GAM-determined allele frequencies at 10 loci and a custom R script employing equation 10 of Rannala and Mountain (1997) at 10 distances along the coast (Table S2). We then assigned each individual larva to the spawning location with the maximum genotype likelihood.

2.4 | Otolith microchemistry analyses

Detailed methods for obtaining microchemistry data from larval otolith cores may be found in Appendix S2. To test the null hypothesis of no difference in natal core microchemistry among larvae ingressing to different estuaries or during different time periods, the effects of ingress site and time period for each elemental ratio (Sr:Ca, Mg:Ca, Mn:Ca, Fe:Ca, Cu:Ca, Cd:Ca, Ba:Ca, Sn:Ca, Pb:Ca and U:Ca) were analysed using a two-way analysis of variance (ANOVA) following $\log_{10}$-transformation. Multivariate analysis of variance (MANOVA) was also used to test for differences in combined larval otolith core trace elements among ingress sites and time periods. Data were scaled and then nonmetric multidimensional scaling (nMDS) was performed using the *nmds* function in the ECODIST version 2.0.1 package (Goslee & Urban, 2007) in R for each time period.

As an additional test of the extent to which larvae ingressing to the same site also shared similar natal core signatures, we performed linear discriminant function analysis (LDA) despite the probable incorrect assumption of a single larval source per ingress location. The

typical use of LDA classifies individuals based on the microchemistry of known locations. This was not our goal. Instead, we tested whether ingress site could be predicted from natal core signatures. If ingress site could be accurately predicted, it would suggest that larvae ingressing to the same estuary had been born near each other at similar natal sites, even though such natal sites were geographically far from the ingress site. If ingress site could not be predicted, it would suggest substantially more mixing between natal sites and ingress sites. We used all 10 scaled elemental ratios and the leave-one-out jackknife procedure in the MASS version 7.3–47 package (Venables & Ripley, 2002) in R. We calculated 68% confidence ellipses for individuals captured from each ingress site using the ELLIPSE version 0.3-8 package (Murdoch & Chow, 1996) in R, and used these 141 individuals as a training data set for LDA to define otolith natal core signatures for larvae ingressing at each site. We then predicted the ingress site of the remaining 56 individuals using the elemental signature at the natal core. Posterior group membership probabilities were also determined.

To avoid making a priori assumptions about group membership, we also performed clustering for each time period separately using all 10 elements. The optimal number of clusters was determined using the *fviz_nbclust* and *NbClust* functions in the FACTOEXTRA version 1.0.5 (Kassambara & Mundt, 2017) and NBCLUST version 3.0 (Charrad, Ghazzali, Boiteau, & Niknafs, 2014) packages, respectively, in R. The optimal number of clusters then informed k-means clustering using the *kmeans* function in the STATS package.

2.5 | Assignment and exclusion of larval groups using otolith microchemistry and genetics

For larvae with both otolith microchemistry and genetic data, we utilized both kinds of data to further investigate larval origins, rather than relying on either data type alone. Because larvae dispersed from the continental shelf to the ingress estuary in which they were captured, we used otolith microchemistry at the natal core to cluster individuals that were likely spawned together in the same offshore water mass. We then assigned natal origins to the clusters of larvae using genetic assignment and exclusion tests. We utilized groups of larvae and pooled genotype data for increased assignment accuracy (Baudouin, Piry, & Cornuet, 2004).

First, we subset the data by time period (early, middle and late) and performed clustering in the same fashion as for the larvae with only otolith microchemistry data.

Second, we used genetic assignment and exclusion tests to determine the natal origins of clustered larvae. For assignment, we calculated the observed likelihood that each cluster originated from each of the 10 potential spawning reference locations using the GAM-determined allele frequencies at the 10 candidate loci (Rannala & Mountain, 1997). We assigned each cluster to the spawning location with the maximum-likelihood method.

For the exclusion method, we used a Monte Carlo resampling method that employs allele frequencies from reference locations (Cornuet et al., 1999; Rannala & Mountain, 1997) to generate distributions of the likelihood criteria that each larval cluster originated from a given spawning location. For comparison against a given cluster composed of Z individuals, we randomly constructed Z genotypes from the allele frequencies in each of the 10 reference locations at each of the 10 loci in adult summer flounder (Table S2). We repeated this 10,000 times to produce the expected distributions of likelihood values for Z individuals that originated in each of the 10 potential locations of origin. For each cluster, the resampled distribution of likelihood criteria was compared to the corresponding observed genotype likelihood for each reference location, and the probability that the cluster of individuals originated from the reference location was calculated as the proportion of resamples with genotype likelihoods less than the observed value (Cornuet et al., 1999). Unlike the assignment method, this method allowed us to calculate a measure of confidence that a cluster of larval individuals originated from a potential spawning location. Similar to Berry et al. (2004), each cluster of larval individuals was assigned to or excluded from potential source locations in three ways. Individuals were (a) assigned to the most likely location, (b) excluded from all locations with ≥80% confidence of exclusion ($p \leq .20$), and (c) excluded from all locations with ≥95% confidence of exclusion ($p \leq .05$).

To examine whether our data set had evidence of siblings dispersing together, we conducted an exploratory sibship analysis with COLONY (Jones & Wang, 2010). No siblings were detected and the analysis was not pursued further.

3 | RESULTS

3.1 | Genotyping results

The average number of quality-filtered reads per individual was 868,180 ± 811,927 (mean ± *SD*). Mapping to our reference assembly resulted in average coverage of 13×. Variant calling across larvae and adults identified 422,767 putative SNPs, and of these, 1,904 loci with an average read depth of 71× across 293 larvae passed filtering.

3.2 | Population structure

A PCA suggested that larval summer flounder were genetically similar across space and time at a genome-wide scale (Figure S2), and these results were confirmed using AMOVA, regardless of hierarchical level (Tables S3 and S4). Pairwise F_{ST} values between ingress site and time period groups were generally quite small (−0.0016 to 0.0019), except for those including the one larva from North Inlet, SC (Table S5).

After testing $K = 1$ to $K = 5$ in STRUCTURE, the mean likelihood value ($L(K)$) and the Evanno method (ΔK) indicated $K = 1$ and $K = 2$ clusters for the full data set containing 1,904 loci, respectively. We interpret these results as a lack of population structure in larvae because all individuals were admixed at approximately the same proportions, regardless of the K value (Figure S3).

3.3 | Individual assignment using genetics

Simulated individual larval genetic assignment using the 10 candidate outlier loci revealed weak resolution for assigning individuals back to location of origin, but with greatest confidence for larvae originating in the extreme northern (A) and extreme southern (J) locations (Figure 2a; ~47% accuracy in both cases). Our ability to assign individual larvae was limited because genotype likelihoods were quite similar between potential source locations (Figure S4). However, simulated individuals drawn from north (A–E) or south

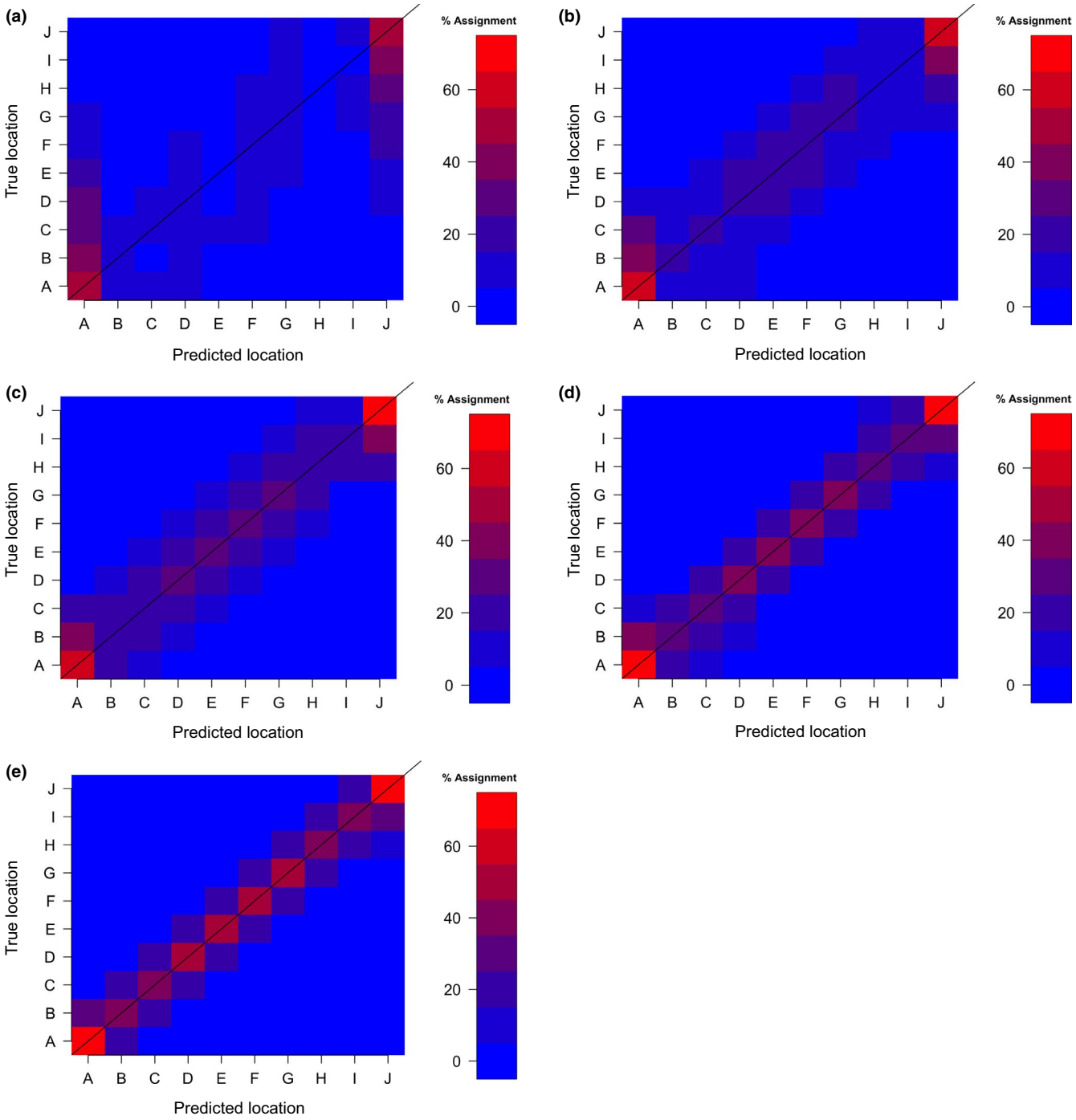

FIGURE 2 Increased assignment accuracy as the number of individuals used for assignment increased. Alleles of (a) one individual, (b) five individuals, (c) 10 individuals, (d) 20 individuals, and (e) 30 individuals were simulated from each of 10 locations (A–J) using the 10 allele frequencies in Table S2. Genotype likelihoods were calculated for individuals or groups of individuals for each spawning location, and these were then assigned to the most likely location. The percentage of correct assignments increased and coalesced around the 1:1 line as the number of individuals increased [Colour figure can be viewed at wileyonlinelibrary.com]

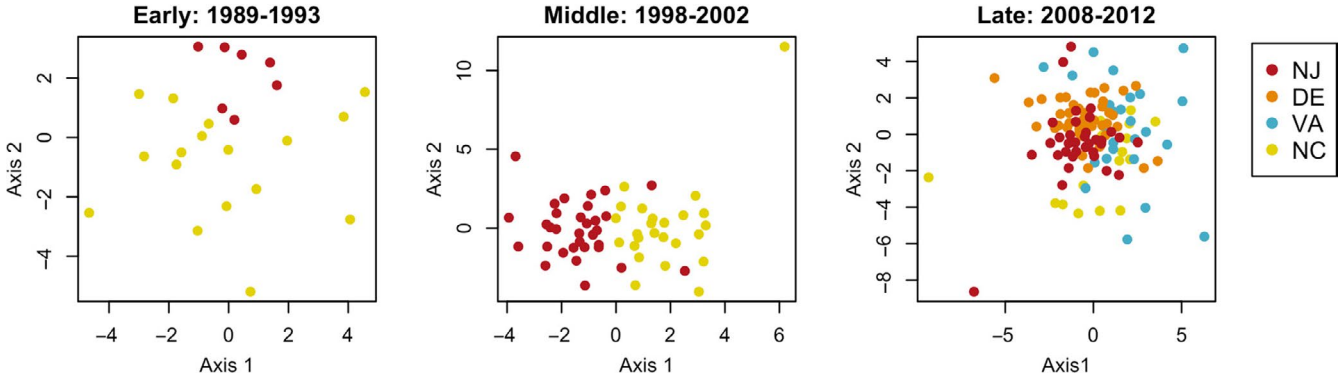

FIGURE 3 Nonmetric multidimensional scaling (nMDS) plots for summer flounder larvae caught within each time period using 10 elemental otolith microchemistry ratios (Sr, Mg, Mn, Fe, Cu, Cd, Ba, Sn, Pb and U) relative to Ca. Segregation between ingress sites located north (NJ: New Jersey; and DE: Delaware) and south (VA: Virginia; and NC: North Carolina) of Cape Hatteras was visible through time [Colour figure can be viewed at wileyonlinelibrary.com]

(F–J) of Cape Hatteras, NC, were usually assigned back to the correct north (~57%–78% accuracy) or south side of Cape Hatteras (~53%–79% accuracy; Figure 2a).

Across all time periods, observed larvae ingressing to Little Egg Inlet, NJ, Roosevelt Inlet, DE, and York River, VA, were equally likely to be assigned back to sources north and south of Cape Hatteras (52% vs. 48% for NJ; 56% vs. 44% for DE; and 46% vs. 56% for VA, respectively). The majority of larvae ingressing to Beaufort, NC (69%), were assigned to sources south of Cape Hatteras and the individual from North Inlet, SC, probably originated from the southern-most (J) reference location (Figure S5).

3.4 | Otolith microchemistry

Otolith microchemistry resulted in high-quality data for 197 larval individuals. Otolith core microchemistry varied significantly among larvae ingressing to different estuaries for Mg, Mn, Fe, Ba and Sn (two-way ANOVA across ingress site and time period, $p < .01$; Table S6, Figure S6) and over time for Mg and Pb (two-way ANOVA, $p < .01$; Table S6, Figure S7). The combined elemental signatures at the natal otolith core differed significantly among ingress sites (MANOVA: Pillai's trace = 0.929, $F_{3,193} = 8.35$, $p < .0001$) and time periods (MANOVA: Pillai's trace = 0.368, $F_{2,194} = 4.199$, $p < .0001$). Segregation between ingress sites located north (NJ and DE) and south (VA and NC) of Cape Hatteras was evident in the nMDS based on otolith core microchemistry within each time period (Figure 3).

When no a priori assumptions about group membership were made, clustering of larvae based on otolith microchemistry data revealed that many clusters were composed of larvae that ingressed either to the same estuary or to adjacent estuaries (Figure S8). Even with the likely incorrect assumption of a single larval source per ingress location, LDA also showed that individuals captured at an ingress site had natal signatures characteristic of other larvae captured at the same ingress site, suggesting that they were spawned in roughly similar locations (Figures S9 and S10), even when LDA was performed for each time period separately (Figure S11). In reality, ingress sites probably include larvae from multiple natal sources. Mg, Mn, Fe and Sn drove many of the patterns observed in LDA classification.

3.5 | Cluster assignment and exclusion using otolith microchemistry and genetics

In contrast to individual larval assignments, we found greatly improved accuracy when assigning clusters of larvae identified through shared elemental signatures, especially as the size of the larval clusters increased (Figure 2; Figure S4). Multiple k-means clustering techniques determined that the optimal number of larval clusters was six, two and three for the early, middle and late time periods, respectively (Figure S12).

When clusters were assigned to the most likely reference location, eight of the clusters (73%) were assigned to the reference locations nearest Cape Hatteras, NC (Locations E and F). The remaining three clusters were assigned to Location C or Location G (Figure 1; Table 2). In particular, the method had confidence in the assigned origins of the larger clusters from the middle and late time periods (Figure 2d,e).

Larvae ingressed to estuaries both close to and far from their most likely location of origin. For example, individuals in clusters E1, E5 and E6 all ingressed at Beaufort, NC, and were most likely to originate from the reference locations closest to Beaufort, NC (Locations F and G). In contrast, some individuals in cluster M2 ingressed to Little Egg Inlet, NJ, but probably originated off southern Virginia (Location E; Figure 4).

The assignment results revealed substantial dispersal across the putative biogeographical break at Cape Hatteras. For example, the majority of individuals in cluster M2 ingressed at Beaufort, NC, but were most likely to originate from the reference location just north of Cape Hatteras, NC (Location E; Figure 4). In addition, the majority of individuals in cluster L1 ingressed at Roosevelt Inlet, DE, and Little Egg Inlet, NJ, but were most likely to originate south of Cape Hatteras, NC (Location F; Figure 4). The assignment results

TABLE 2 Larval summer flounder cluster assignment and exclusion results from each time period. Clusters were assigned to the most likely (ML) spawning reference location along the coast (see Figure 1). Clusters were also excluded from potential reference locations at two significance levels: .20 and .05

	Size (n = 151)	Location A (north)	Location B	Location C	Location D	Location E	Location F	Location G	Location H	Location I	Location J (south)
Early (1989–1993)											
E1	n = 4						ML				
E2	n = 3					ML					
E3	n = 4					ML			$p < .20$	$p < .20$	$p < .20$
E4	n = 4			ML							
E5	n = 3							ML			
E6	n = 3							ML			
Middle (1998–2002)											
M1	n = 21					ML					
M2	n = 26					ML					
Late (2008–2012)											
L1	n = 33						ML				
L2	n = 22					ML	$p < .20$	$p < .20$	$p < .20$	$p < .05$	$p < .05$
L3	n = 28						ML				

suggest that exchange of larval summer flounder throughout the species range is common, frequently extends across Cape Hatteras and sometimes occurs in the direction opposite the dominant Gulf Stream.

The exclusion method suggested that clusters E3 and L2 had low probabilities ($p < .20$) of originating from southern reference locations and could therefore be excluded with high confidence (Table 2). These results supported and were consistent with the assignment results. Other clusters could not be confidently excluded from particular reference locations.

4 | DISCUSSION

Natural history collections, and natural tags intrinsic to preserved specimens, are useful for investigating a wide range of ecological and evolutionary processes (Webster, 2018). We utilized intragenerational otolith microchemistry and intergenerational genetic markers, separately and in combination, from archived larval summer flounder captured across a quarter of a century to reveal that contemporary dispersal during the larval stage is sufficiently widespread to result in extensive population mixing along the US East coast. Neither genetics nor otolith microchemistry alone were adequate for identifying the origins of larval fish because allele frequencies were homogeneous among source locations and because elemental signatures at potential spawning locations could not be validated. However, natal origins could be identified with greater accuracy when data from candidate loci and otolith microchemistry were combined in an integrated approach. We found that many larvae were most likely to originate in the vicinity of Cape Hatteras, larvae dispersed both near and far from their site of origin, and dispersal sometimes occurred in the opposite direction to the northward-flowing Gulf Stream.

4.1 | Single- and multimarker approaches to infer dispersal

Genetic assignment tests are widely used to determine the population of origin for an individual or a group of individuals in order to infer dispersal rates, identify immigrants, recognize hybridization events or classify the proportion that each source population contributed to a mixture of individuals (Manel et al., 2005). However, the utility of these methods may be reduced when effective population size is large and genetic differentiation is weak (Allendorf, Hohenlohe, & Luikart, 2010; Berry et al., 2004), as is often the case for many species (Waples, 1998; Ward, Woodwark, & Skibinski, 1994), including summer flounder (Hoey & Pinsky, 2018). Low genetic differentiation at neutral markers has limited efficacy for population assignment because of ongoing gene flow. However, candidate outlier loci, or gene-associated markers, that arise due to divergent environmental selection are promising for population assignment because of their elevated differentiation compared to neutral markers (Benestan et al., 2015; DeSaix et al., 2019; Freamo et al.,

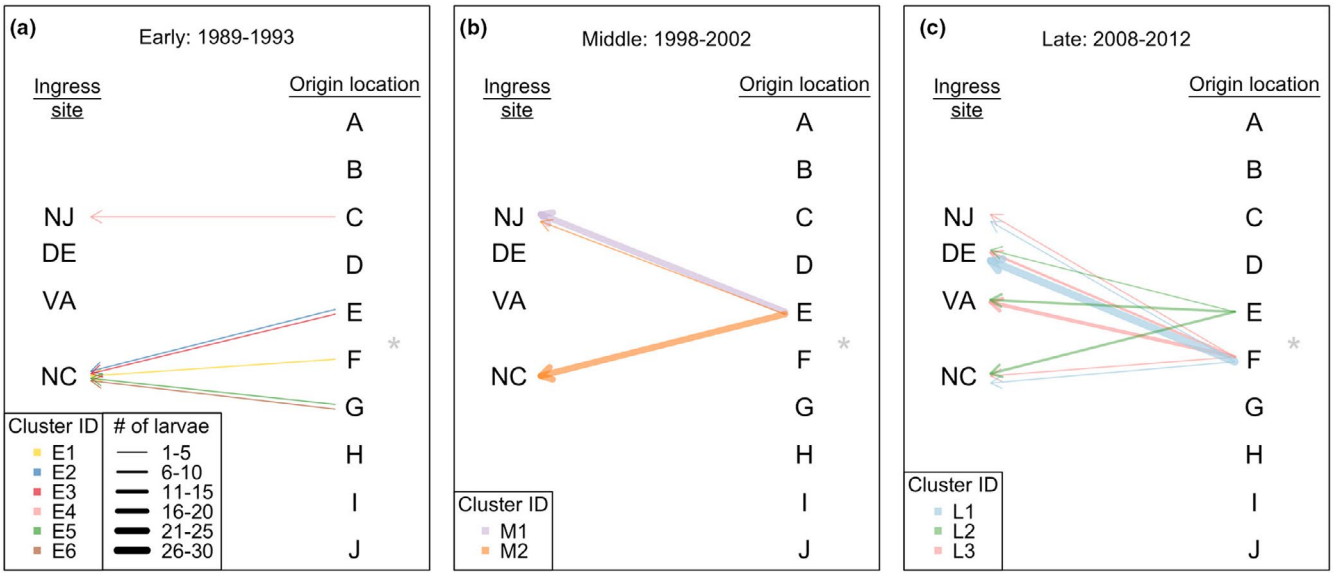

FIGURE 4 Schematic depicting the probable origin on the continental shelf (A–J; ordered from north to south, see Figure 1) and estuarine destination of dispersing summer flounder larvae. The width of the arrow indicates how many larvae ingressed to sites (NJ: New Jersey, DE: Delaware, VA: Virginia, NC: North Carolina) in the (a) early (1989–1993), (b) middle (1998–2002) and (c) late (2008–2012) time periods. Each cluster of larvae is represented by a unique colour. The location of Cape Hatteras, a biogeographical break for many marine species, is indicated by a grey asterisk [Colour figure can be viewed at wileyonlinelibrary.com]

2011; Nielsen et al., 2009, 2012). Genetically diverged candidate loci contain higher information content, allowing greater assignment accuracy than would be possible using an equal or greater number of neutral loci. For example, Freamo et al. (2011) achieved 85% assignment accuracy when using 14 candidate outlier loci compared to 75% assignment accuracy when using 67 neutral loci in salmon, and studies in other systems have achieved equal success using many more markers (Benestan et al., 2015; DeSaix et al., 2019; Rannala & Mountain, 1997). The 10 candidate outlier loci available for summer flounder were differentiated across geography, but only weakly so, which resulted in low power to assign individuals to populations of origin. Identification of additional candidate outlier loci would probably improve our power to distinguish the origins of individual larval summer flounder when using only genetic assignment.

Otoliths are useful for studying dispersal and population connectivity within individual lifespans of fishes (Thorrold et al., 2001). The conventional use of otolith microchemistry for population assignment requires that a chemical atlas can be accurately created, typically by capturing individuals at a location that corresponds to the otolith section being studied (i.e., captured at the natal site when analysing the otolith core). By capturing individuals from all known natal locations, a reference elemental atlas is developed to which other individuals can be assigned (Gillanders, 2002; Shima & Swearer, 2009). As a result, these studies are often limited to species spawned in shallow bays and estuaries where water chemistry differences are greatest. However, many marine species are spawned in more open, coastal environments, often resulting in a temporal disconnect between the natal core and the individual's capture location. Chemical atlases are difficult to recreate for such species, meaning that for summer flounder, otolith microchemistry alone could not be used to explicitly define the specific natal location(s). Rather, otolith microchemistry indicated that ingressing summer flounder larvae spanned a range of natal environmental signatures. Some groups of larvae were spawned in the same offshore location and dispersed together, but mixing along the coast was also evident. We note that traditional assignment of summer flounder natal sources using otolith microchemistry could potentially be achieved using several long-term collections of ichthyoplankton on the continental shelf, including the Marine Resources Monitoring, Assessment, and Prediction (MARMAP) and the Ecosystem Monitoring (EcoMon) programmes (Richardson, Hare, Overholtz, & Johnson, 2010; Walsh, Richardson, Marancik, & Hare, 2015), which would be useful to corroborate our results.

In many cases, neither microchemistry nor genetics alone are particularly helpful for assigning individuals to source populations (Cornuet et al., 1999; Gillanders, Sanchez-Jerez, Bayle-Sempere, & Ramos-Espla, 2001; Manel et al., 2005). In this paper, we instead combined both natural tags together to resolve natal sources in a more spatially explicit context and to achieve greater power for assignment. We first clustered individuals from each time period using elemental microchemistry (Tanner, Vasconcelos, Cabral, & Thorrold, 2012), and then used allele frequencies at spatially differentiated candidate loci for genetic population assignment. In doing so, we were able to account for baseline differences in elemental chemistry within each time period while concurrently taking advantage of increased power for genetic assignment of groups (Baudouin et al., 2004) despite relatively few genetic markers. Assignment validation indicated particularly robust results when clusters were composed

of ~20 individuals or more, which was true of all our clusters except those from the earliest time period. Our combined multimarker approach allowed for a higher resolution understanding of larval dispersal along the US East coast over a quarter of a century than would otherwise be possible using a single marker. Similar approaches are likely to be useful for future connectivity studies of coastally spawned species.

4.2 | Dispersal across biogeographical breaks

Due to the positive correlation between pelagic duration and dispersal distance (Shanks, 2009), marine populations were once thought to be highly connected and well-mixed, but recent investigations of realized population connectivity in the sea have documented much more limited dispersal (Almany et al., 2017; Baetscher et al., 2019; Jones et al., 2005; Palumbi, 2003). Biogeographical breaks are areas where physical processes create sharp physical and biochemical discontinuities. Such discontinuities occur in all oceans and are thought to limit larval exchange (Galarza et al., 2009). Thus, biogeographical breaks provide interesting and important opportunities to understand dispersal scales in the ocean (Cowen, Paris, & Srinivasan, 2006). We tested for larval summer flounder dispersal across Cape Hatteras, a known biogeographical break for a variety of other invertebrates and fish (Baker et al., 2008; Briggs, 1974; Roy et al., 2012), and a purported barrier for summer flounder (Kraus & Musick, 2001; Wilk et al., 1980). However, we found larval dispersal to be bidirectional across Cape Hatteras, suggesting that Cape Hatteras does not function as a strong biogeographical barrier to movement for summer flounder larvae. We also found that larval summer flounder ingressed to locations both near and far from their most likely origin (~100–500 km), providing empirical evidence for substantial connectivity across space in the sea.

Both biological characteristics of summer flounder and oceanographic processes probably influenced the high population connectivity we observed. Summer flounder are highly fecund and exhibit serial spawning across an extended season, although peak spawning coincides with the autumn breakdown of the thermocline and the resulting plankton bloom (Morse, 1981). Northward movement across Cape Hatteras could be achieved if larvae were entrained in the Gulf Stream and subsequently concentrated and transported back west across the continental shelf via warm core rings (a type of mesoscale eddy; Hare et al., 2002; Hare & Cowen, 1996) or filaments between mesoscale eddies (Harrison, Siegel, & Mitarai, 2013) before ingress. The southwest propagation of warm core rings (Auer, 1987) and the southerly flow of shelf and slope waters due to the Labrador Current (Bumpus, 1973) could facilitate southward dispersal of larvae spawned in the Mid-Atlantic Bight, with flux across Cape Hatteras occurring via wind or buoyancy-driven intrusions (Grothues et al., 2002; Stegmann & Yoder, 1996). Biological characteristics, such as iteroparity and considerable larval production, may also interact with physical oceanographic features to increase retention and upstream spread of larval summer flounder in the Mid- and South Atlantic Bights (Byers & Pringle, 2006).

4.3 | Dispersal over time: Implications, assumptions and opportunities

Empirical estimates of dispersal over time are crucial for validating our theoretical understanding of the variable nature of larval dispersal and its consequences (Siegel et al., 2008) and for improving fisheries management (Fogarty & Botsford, 2007). An increasing number of studies have examined dispersal over intraseasonal (Cook, 2011) and interannual (Hogan, Thiessen, Sale, & Heath, 2012; Kraus & Secor, 2005; Reis-Santos et al., 2013) scales, but dispersal estimates for more than two cohorts and over decadal scales are rare. Similar to our theoretical understanding, these empirical studies indicate that dispersal distances, local retention and patterns of larval connectivity can be quite variable over time. In summer flounder, we also found variation in dispersal patterns, with some estuaries receiving more larvae from particular spawning locations in certain time periods. However, we found instances of larval summer flounder originating both near and far from their ingress site, and this phenomenon appeared regularly in each of three time periods examined over a 24-year time frame. Our finding of a high degree of larval connectivity over decadal timescales in summer flounder suggests that larval dispersal is frequent and extensive enough to result in genetic near-homogeneity.

The utility of spatially divergent loci for genetic population assignment with temporally spaced samples is dependent on allele frequency differences being stable over time. Allele frequencies at candidate loci may be difficult to detect or may fluctuate over time due to the transient nature of small-effect loci (Yeaman, 2015) or spatially varying environmental selection acting in each generation (Bernatchez, 2016; Hedrick, Ginevan, & Ewing, 1976). However, the majority of our candidate outlier loci in adult summer flounder were associated with bottom temperature, and a strong thermal gradient due to the Gulf Stream exists along the US East coast (Briggs, 1974). Recent evidence points to a weakening of the Atlantic meridional overturning circulation , of which the Gulf Stream is an important element, as well as a northward shift of the Gulf Stream, and that these changes are probably due to climate change (Caesar, Rahmstorf, Robinson, Feulner, & Saba, 2018; Thornalley et al., 2018). This is reflected in the increased occurrence of southern species at the Little Egg Inlet, NJ, site (Morson, Grothues, & Able, 2019). Despite these changes, the persistence of a thermal gradient suggests that selection for temperature-associated genetic markers has existed over the last few decades as well.

Dispersal may promote or constrain adaptive divergence across an environmental landscape (Garant, Forde, & Hendry, 2007; Lenormand, 2002). Within the context of rapid environmental change, high connectivity over time may be particularly advantageous because it increases adaptive potential through beneficial gene flow. With a baseline understanding of dispersal in summer

flounder, ongoing and future dispersal will be easier to evaluate. We took advantage of existing natural history collections—invaluable, long-term data sets of life's diversity (Lister & Climate Change Research Group, 2011)—to retroactively investigate dispersal patterns over time. Such collections have also been key to investigating phenotypic (Cross, Harper, & Peck, 2018), phenological (Primack, Imbres, Primack, Miller-Rushing, & Tredici, 2004) and distributional shifts (Moritz et al., 2008), further highlighting the importance of archived collections for studying ecological and evolutionary patterns and processes. Furthermore, these studies illustrate the need for long-term sampling programmes to make connections with governmental, academic or private (e.g., open nonprofit) research collections so that specimens resulting from their sampling can be catalogued, curated and made available (and known) to the broader research community (Singer, Love, & Page, 2018). Appropriately preserved specimens not only serve as historical baselines by documenting biogeography and morphology in space and time, but also harbour a wealth of information in the form of genetic, biochemical and geochemical natural tags, which themselves can be time capsules of information. However, these collections are most useful when accessible and available to the research community and properly cared for in perpetuity. The nature of our summer flounder specimens allowed for a combined otolith–genetic approach, identifying instances of bidirectional dispersal both near and far from sites of origin across an environmental gradient and over time. Future studies combining contemporary specimens and those available in natural history collections have great potential to reveal how historical and ongoing environmental changes have and will continue to impact the ecological and evolutionary trajectory of organisms (Johnson et al., 2011; Meineke et al., 2019; Webster, 2018), if we can use collections in innovative and synergistic ways and revisit them as new approaches are developed to extract information.

4.4 | Consequences for summer flounder management

Summer flounder support economically important commercial and recreational fisheries, especially in the Mid-Atlantic where biomass is highest (Packer et al., 1999), and our findings have important implications for summer flounder biology and conservation. The results from our multimarker approach indicate that the majority of larvae probably originated in the vicinity of Cape Hatteras. The consistency with which larval clusters were assigned back to the Cape Hatteras region through time was striking, despite the northward shift of summer flounder populations in recent decades (Bell, Richardson, Hare, Lynch, & Fratantoni, 2014; Nye, Link, Hare, & Overholtz, 2009). Even though the majority of summer flounder are thought to spawn from Cape Cod, MA, to Cape Lookout, NC, along the continental shelf (Able & Fahay, 1998; Smith, 1973), our data suggest that spawning adults in the vicinity of Cape Hatteras comprise a particularly important source of production and contribute disproportionately to coastwide annual recruitment. The Cape Hatteras region could be targeted for spawning stock protection if management needs warranted. In addition, shared dispersal trajectories may have demographic consequences by affecting the distribution of phenotypes in the subsequent life stages (Shima & Swearer, 2016). Further research using samples captured as soon as possible after spawning (i.e., ichthyoplankton samples captured offshore), additional adult samples, larvae that ingressed to estuaries not sampled in this study and/or other natural and artificial tags would be useful to further ground-truth our findings and confirm if individuals spawned in the vicinity of Cape Hatteras contribute disproportionally to the next generation. Additionally, the use of biological–oceanographic (biophysical) models would be helpful for understanding how larvae spawned throughout the species' range disperse along the coast.

5 | CONCLUSIONS

We demonstrated how an integrated multimarker approach can improve estimates of contemporary dispersal, and how natural history collections can greatly extend the temporal scale of investigations examining ecological and evolutionary processes. By using natural tags that span intra- and intergenerational timescales, our combined approach enabled a higher resolution understanding of the magnitude and directionality of dispersal over time. Our results provide direct evidence of high connectivity in a marine species, and contrasts with recent evidence of high self-recruitment in the sea. High dispersal over space and time appears to be quite common in some marine species and may be a particularly important mechanism maintaining genetic diversity and evolutionary potential in populations during an era of rapid environmental change.

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AUTHOR CONTRIBUTIONS

J.A.H., M.L.P., K.W.A. and F.J.F. designed the study; K.W.A., E.J.H., G.T.K., T.E.T., J.C.T., J.A.H. and F.J.F. obtained and organized the samples; J.A.H. prepared the ddRADseq libraries, performed the bioinformatics and analysed the cleaned genetic data set; F.J.F. and Q.A.W. dissected and performed laser ablation inductively coupled plasma mass spectrometry on cleaned larval otoliths; J.A.H. and F.J.F. analysed elemental ratios; J.A.H., M.L.P. and F.J.F. designed analysis methods; all authors discussed the results; J.A.H. wrote the manuscript; all authors edited the manuscript.

DATA AVAILABILITY STATEMENT

Raw sequencing reads are archived in the NCBI Sequence Read Archive (SRA) database (accession no. PRJNA600652). Other data and code associated with this study are available through Zenodo at https://doi.org/10.5281/zenodo.3670955(Hoey et al., 2020).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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